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ScienceDirect
Ecotoxicology and Environmental Safety 72 (2009) 479–489
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Biomonitoring of cyanotoxins in two tropical reservoirs by cladoceran toxicity bioassays ☆

Aloysio da S. Ferrão-Filho[a,*], Maria Carolina S. Soares[b], Valeria de Freitas Magalhães[c], Sandra M.F.O. Azevedo[c]

[a]*Laboratório de Avaliação e Promoção da Saúde Ambiental, Departamento de Biologia, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil 4365, Manguinhos, Rio de Janeiro, RJ-CEP 21045-900, Brazil*
[b]*Departamento de Botânica, Museu Nacional, Universidade Federal do Rio de Janeiro, São Cristovão, Rio de Janeiro, RJ 20940-040, Brazil*
[c]*Instituto de Biofísica Carlos Chagas Filho, CCS, Bloco G, Universidade Federal do Rio de Janeiro Ilha do Fundão, Rio de Janeiro, RJ 21949-900, Brazil*

Received 19 June 2007; received in revised form 17 January 2008; accepted 12 February 2008
Available online 14 April 2008

Abstract

This study evaluates the potential for the use of cladocerans in biomonitoring of cyanobacterial toxins. Two zooplankton species (*Daphnia gessneri* and *Moina micrura*) were cultivated in the laboratory for use in acute (48 h) and chronic (10 days) bioassays. Water samples were collected from two reservoirs and diluted in mineral water at four concentrations. Survivorship in the acute bioassays was used to calculate LC_{50}, and survivorship and fecundity in chronic bioassays were used to calculate the intrinsic population growth rate (r) and the EC_{50}. Analysis of phytoplankton in the water samples from one reservoir revealed that cyanobacteria were the dominant group, represented by the genera *Anabaena*, *Cylindrospermopsis*, and *Microcystis*. Results of bioassays showed adverse effects including death, paralysis, and reduced population growth rate, generally proportional to the reservoir water concentration. These effects may be related to the presence of cyanobacteria toxins (microcystins or saxitoxins) in the water.

Keywords: Cyanobacteria; Cyanotoxins; Zooplankton; Cladocera; Seston; Toxicity bioassays

1. Introduction

The use of aquatic organisms for biomonitoring (USEPA, 1984) is an important tool in aquatic ecotoxicology, allowing the evaluation of the potential toxicity of substances from anthropogenic origin (xenobiotics) as well as from living organisms (toxins). The cladoceran of the genus *Daphnia* has been well established as a useful model organism in ecotoxicology, for investigating the impact of toxic substances in freshwater (Baird et al., 1989; Gersich and Milazzo, 1990; Diamantino et al., 2000; Guilhermino et al., 2000) with several standardized protocols in international environmental agencies (Biesinger et al., 1987; Persoone and Van de Vel, 1988; ASTM, 1994; Weber, 1993). In Brazil, the Brazilian Association of Technique Norms established the first protocol with *Daphnia similis* (ABNT, 1993), a temperate species, and more recently with *Ceriodaphnia silvestri*, a native species (ABNT, 2003). However, protocols for other tropical cladocerans, which are common representatives of our water bodies, remain to be developed. There are not protocols either for testing the specific effects of cyanotoxins in freshwater cladocerans.

Cyanotoxins are toxins produced by many genera of cyanobacteria and are commonly referred as hepatotoxins and neurotoxins (Chorus and Bartram, 1999). Hepatotoxins are mainly represented by cyclic peptides (microcystins (MCs) and nodularins) and alkaloids (cylindrospermopsin), and neurotoxins by alkaloids (anatoxina-a, anatoxina-a(s) and saxitoxins (STX)). Several cases of poisoning of

☆ Funding: This research was supported by the Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro-FAPERJ (Proc. # E26/150.705/2002). The authors assure that any studies involving humans or experimental animals were conducted in accordance with national and institutional guidelines for the protection of human subjects and animal welfare.
*Corresponding author. Fax: +55 21 2560 6474.
E-mail address: aloysio@ioc.fiocruz.br (A.S. Ferrão-Filho).

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doi:10.1016/j.ecoenv.2008.02.002

wild and domestic animals, and also humans, involving cyanotoxins have been reported worldwide (Jochimsen et al., 1998; Chorus and Bartram, 1999; Azevedo et al., 2002). Thus, cyanotoxins represent a risk to human health, especially when consumed in drinking water or through the food.

Zooplankton has been considered one of the main targets of cyanotoxins, due to the evolutionary role of such toxins as a chemical defense against grazing (Lampert, 1981), and because zooplankton can be directly exposed to cyanotoxins through their diet with the ingestion of cyanobacterial cells. As cyanotoxins have specific effects on these microcrustaceans (DeMott et al., 1991; Ferrão-Filho et al., 2000, 2008; Ferrão-Filho and Azevedo, 2003), they are excellent biomonitors for these toxins in marine as well as in freshwater systems. Also, changes in the zooplankton community structure, such as a shift from large crustaceans like cladocerans and copepods to a community dominated by small cladocerans and microzooplankton, may reflect the ecological impact of cyanobacteria on aquatic communities (Leonard and Pearl, 2005).

Zooplankton can also accumulate cyanotoxins and act as vectors of these toxins through the food chain (Kotak et al., 1996; Thostrup and Christoffersen, 1999; Ferrão-Filho et al., 2002; Ibelings et al., 2005), making them suitable for studying trophic transfer of these toxins to higher trophic levels. Knowledge about which species are more resistant to cyanotoxins, and more likely to transfer these toxins, would help to develop predictive models for the risk assessment of the exposure of higher trophic levels through the accumulation of cyanotoxins in the food chain.

The aim of this study was to evaluate the potential for the use of cladocerans in biomonitoring of tropical eutrophic reservoirs, focusing specifically on the effects of cyanobacterial toxins. For this purpose two approaches were used: (1) acute and (2) chronic static-renewal bioassay, using cladocerans isolated from an oligo-mesotrophic reservoir (Lajes), which have never been previously exposed to cyanobacterial blooms.

2. Material and methods

2.1. Study areas

Lajes reservoir is located near Barra do Pirai town, Rio de Janeiro State (Fig. 1). It was built in 1905–1908 for electricity generation purposes, but it is also used for drinking water supply. Its drainage basin is well preserved, having well-built marginal vegetation, being considered an oligo-mesotrophic system. Cyanobacterial blooms have never been reported in this reservoir, and the main cyanobacteria species have not been known as toxin producers. A fishing farm project with *Tilapia rendalli* has been carried out in this reservoir during last 5 years, and there is concern that it can degrade water quality. Due to this concern, this reservoir was included in this biomonitoring program.

Funil reservoir is located near Resende town, Rio de Janeiro State (Fig. 1), and it is situated on the Paraíba do Sul river valley. This reservoir

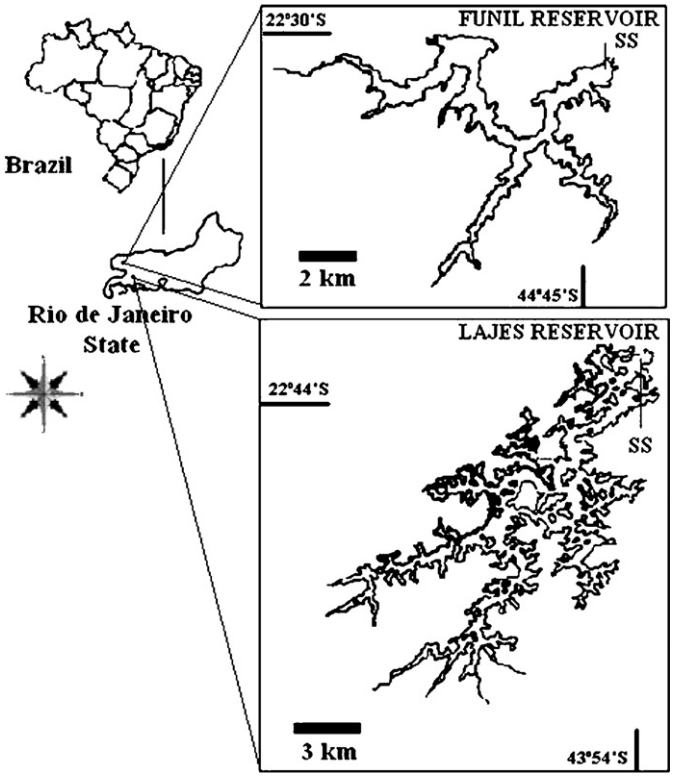

Fig. 1. Location of Funil and Lajes reservoirs and the sampling stations (SS). Limnological characteristics of reservoir are area: 40.0–47.8 km^2, volume: 890×10^6–450×10^6 m^3, average depth: 22–15 m, maximum depth (at the dam): 70–40 m.

was finished in 1969 for water supply and electricity generation purposes. It is also used for recreation, fishing, and for the regulation of floods in the surroundings. Its drainage basin is highly impacted due to erosion processes and discharge of domestic and industrial wastes. The large input of nutrients has turned this reservoir hypereutrophic, with recurrent blooms of cyanobacteria.

Both reservoirs contribute to the water supply of the Rio de Janeiro City and surroundings. These reservoirs have been monitored since 2002 concerning blooms of cyanobacteria in several sampling stations. However, only one sampling site near the dam of both reservoirs was chosen for this biomonitoring program and also because we wanted to know the water quality near the catchment area.

2.2. Cultures of test organisms

Two cladoceran species were isolated from Lajes reservoir in 2002 and were cultivated in the laboratory from parthenogenetic females as clonal cultures for several generations. *D. gessneri* Herbst (adult size: 1.4–1.6 mm; Elmoor-Loureiro, 1997) is reported to occur only in South America, from the Amazonian region to the Southwestern lakes of Brazil (Matsumura-Tundisi, 1984). *Moina micrura* Kurs (adult size: 0.5–1.3 mm; Elmoor-Loureiro, 1997) is a more widely distributed species occurring in both tropical and temperate regions (Rocha et al., 1995). Cladocerans were maintained in commercial mineral water (400–800 mL) plus 20% (100–200 mL) of filtered Funil reservoir water. The mixture with reservoir water was supposed to supply some dissolved organic matter necessary for growth (e.g. vitamins), which is lacking in mineral water. Animals were fed 0.5 mg C L^{-1} of the green alga *Ankistrodesmus falcatus* (Braun). Water medium was renewed three times a week and new cultures were established biweekly.

2.3. Water and phytoplankton samplings

Samples were collected monthly from November 2002 to October 2003 from near the dam of both reservoirs. Water samples were collected by surface grabs and stored in polyethylene bottles on ice. In the laboratory, samples were filtered onto glass fiber filters for retaining the total seston (particles $>1.2\,\mu m$), and the filters were used for carbon and toxin analysis. Water for phytoplankton cell counts were collected using 150 mL amber glass flasks and fixed with Lugols' solution for counting, according to Uthermöhl (1958). The organisms were identified according to the main morphological and morphometric characteristics of the vegetative and reproductive phases. The main phytoplanktonic taxa were identified according to Anagnostidis and Komárek (1985, 1988), Komárek and Anagnostidis (1986, 1998) and Komárek and Komárková (2003). Phytoplankton biovolume ($mm^3\,L^{-1}$) was estimated by multiplying the density of each species by the average volume of its cells, according to Hillebrand et al. (1999), and specific biomass was expressed in mg (fresh weight) L^{-1} (Edler, 1979), assuming a specific density of phytoplankton cells of $1.0\,g\,cm^{-3}$. Concentrated phytoplankton samples were collected using plankton nets of 20 and 70 μm mesh size. The following procedures were performed to concentrate phytoplankton: First, phytoplankton was concentrated using 20 and 70 μm nets in horizontal hauls from a slow-moving boat. Then, in land, samples were submitted to a separation technique using plastic bottles where phytoplankton and zooplankton were separated using carbonated water (water with gas). As colonies and filaments of cyanobacteria tend to float, phytoplankton stayed in the upper layers while zooplankton sank to the bottom of the bottle, where it was removed through a valve. After this procedure, we performed again a sequential filtering of each sample through plankton nets of 300→70→20 μm. With this procedure we obtained mixed phytoplankton samples between 20 and 300 μm. These samples were freeze-dried for toxin analysis.

2.4. Acute and chronic toxicity bioassays

Both tests employed a static-renewal protocol and started with 10 newborns (<24 h old), from the second or third clutch, placed in a flat-bottom test tube filled with 30 mL of water composed of raw water from the Funil reservoir diluted in mineral water, ranging from 0% (control with mineral water) to 100% raw water. Each tube received also the green alga as food at the concentration of $0.5\,mg\,C\,L^{-1}$. A treatment with filtered reservoir water plus green algae was also performed as a control. For the acute bioassays, four replicates per treatment were used. Animals were exposed for 48 h to all treatments and survivors were counted and transferred to new seston suspensions after 24 h. Lethal concentration after 48 h (LC_{50}-48 h) was calculated, whenever possible, using probit analysis through the statistical package SPSS (SPSS Inc., Chicago, IL, USA). For the chronic toxicity bioassays, ten replicate tubes were used. Survivors were transferred every day to new seston suspensions and the age at first reproduction and the number of newborns produced were registered. The chronic bioassays lasted about 10 days, or at least until four clutches were produced by the control group. Age-specific survivorship (l_x) and fecundity (m_x) were used to calculate the population increase rate (r) using the bootstrap technique (Taberner et al., 1993). Effective concentration (EC_{50}), defined as the concentration capable of reducing r to 50% of the control value, was calculated, whenever possible, using probit analysis.

2.5. Chemical analysis

Aliquots of 100–500 mL of the water samples were filtered onto glass fiber filters for carbon analysis of seston by the dichromate–sulfuric acid method (Strickland and Parsons, 1972). MCs in total seston and net phytoplankton were analyzed by filtering 2 L of water onto glass fiber filters or using lyophylized material, respectively. Samples were extracted three times with buthanol:methanol:water (5:20:75 v/v). The extract was evaporated to 1/3 of its initial volume and then passed through a C18 cartridge ($500\,mg\,6\,mL^{-1}$ Bond-Elut, Varian). This cartridge was washed and eluted with distilled water, aqueous methanol 20%, and then with methanol 50%. This last fraction was dried and stored at −20 °C for subsequent analyses. The MCs analysis was performed by high-performance liquid chromatography (HPLC) in a Shimadzu/CLASS VP apparatus with UV detector PDA M10A VP, using a Lichrospher 100 RP-18 column (150 mm × 4.6 mm ∅, 5 μm Merck), and a 100 μL loop. Chromatography was conducted in isocratic conditions as follows: mobile phase acetonitrile and 20 mM of acetate ammonium in pH 5.0 (28:72 v/v) for 20 min, at a flux of $1\,mL\,min^{-1}$, UV detection at 238 nm, and the spectrum of absorbance of each peak was analyzed between 190 and 300 nm. A standard of MC-LR (Sigma) was used as an external standard to the quantification of MCs. The concentration of MCs was estimated by the sum of all peak areas presenting an absorption spectrum with 95% of similarity index with the standard MC-LR and was expressed as concentration of MC-LR equivalents, as described in Chorus and Bartram (1999).

STXs were analyzed for total seston and net phytoplankton, both from lyophylized material (2 L of raw water and ~20 mg of phytoplankton, respectively). Seston and phytoplankton samples were extracted with 5 mL of 0.1 N acetic acid for 1 h. After that samples were centrifuged at 10,800g for 10 min and the supernatant stocked in −18 °C until the analysis. All samples were analyzed by HPLC with the post-column derivatization method (Oshima, 1995) in a Shimadzu/CLASS VP apparatus with fluorescence detector (RF-10A XL), adjusted to 330 nm of excitation and 390 nm of emission, using a 20 μL loop, reverse column Merck LC-18 (Lichrocart® 150 mm × 4.6 mm ∅, 5 μm). The mobile phase consisted of 2 mM hepatanosulfonate in 30 mM ammonium phosphate buffer pH 7.1:acetonitrile 100:5 for STX and neoSTX analysis and 2 mM of hepatanosulfonate in 10 mM ammonium phosphate buffer, pH 7.1, for GTXs analysis with a flow rate of $0.8\,mL\,min^{-1}$. The oxidizing reagent used was 7 mM periodic acid in 10 mM sodium phosphate buffer, pH 9.0, and the reaction with a flow rate of $0.4\,mL\,min^{-1}$. The oxidizing reaction was done in a 10 m coil of Teflon tubing at 80 °C. Before the detection, the reaction was interrupted with a 0.5 M acetic acid. Standard solutions of STX, neosaxitoxin (neoSTX), and goniautoxins (GTX 1 and 4 and GTX 2 and 3) were obtained from the National Research Council (NRC), Institute for Marine Biosciences, Canada. Those standards of toxins were used for identification and quantification of toxins present in the sample, and the sum of all peak areas of toxins found was expressed as concentration of STX equivalents.

2.6. Statistical analysis

Regression analysis was performed with data to infer the relationship between survivorship or intrinsic rate of population increase (r) and the concentration of seston (as % of reservoir water) with the purpose to verify if there was a dose–response effect. We applied t-tests for comparing the mean r values between controls and seston treatments, as we had only the mean values, confidence intervals, and standard errors given by the bootstrap analysis.

3. Results

3.1. Phytoplankton, carbon, and toxins

Phytoplankton of Funil reservoir was dominated most of the time by cyanobacteria, reaching peak biomass in the wet–warm season (November–December 2002–February 2003) and in the spring (September 2003), when cyanobacteria comprised ca. 100% of total phytoplankton (Fig. 2). The lowest total biomass occurred in the cold–dry season (April–June 2003). Table 1 shows the percentage of the main phytoplankton groups present in Funil reservoir during the sampling period. Among the cyanobacteria, the

following species occurred: *Anabaena* spp., *Cylyndrospermopsis raciborskii*, *Microcystis aeruginosa*, and *Microcystis botrys*.

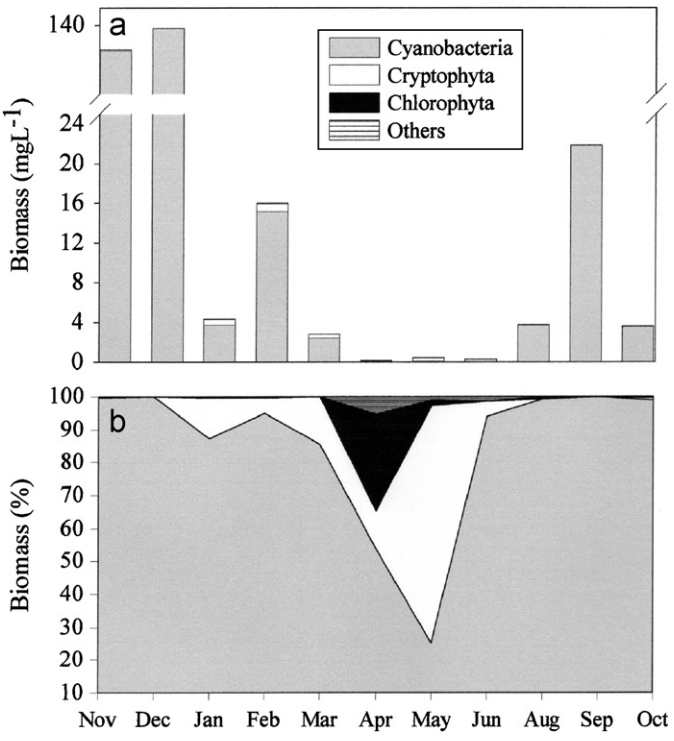

Fig. 2. Phytoplankton biomass expressed as total biomass (a) and percent biomass (b) of each group in Funil reservoir during the bioassays.

Carbon content and toxin concentration in seston and phytoplankton of Funil reservoir are shown in Table 2. Carbon concentrations were higher during the wet–warm season (November–February), coinciding with the peak biomass of phytoplankton, and lower during the cold season (April–June), when phytoplankton biomass was also low. There were also high carbon concentrations and phytoplankton biomass in August and September (spring). MCs were only detected in total seston in November and December, although it was present in high concentrations in net phytoplankton in the whole sampling period. Only a few seston samples were analyzed regarding STXs, due to scarcity of material, and toxins were below the detection limit in these samples. In net phytoplankton, STXs were present in conspicuous concentrations in February, May, and August, and below the detection limit the rest of the sampling period (Table 2).

Although phytoplankton showed reduced biomass during the entire year, green algae, desmids, and cyanobacteria are the dominant groups in Lajes reservoir. No toxins were found in samples from Lajes reservoir.

3.2. Acute bioassays

Acute toxic effects were detected only in *M. micrura* in the wet–warm season (November–December 2002), where survivorship was decreased proportionally to the concentration of raw water from Funil reservoir (Fig. 3). Values of LC_{50} (48 h) in those months were 91.2% and 79.4% of Funil raw water, respectively. *D. gessneri* was not tested in

Table 1
Percentage of phytoplankton groups (as biomass) in Funil reservoir

Genus	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Aug	Sep	Oct
Anabaena	37.6	2.2	0.0	20.3	86.1	0.0	58.0	0.0	32.6	1.3	0.0
Cylindrospermopsis	6.0	1.7	97.1	76.9	13.6	0.0	33.3	0.0	15.5	0.0	0.8
Microcystis	57.0	96.0	2.8	2.8	0.0	18.1	8.7	100.0	44.9	91.0	95.6
Others	0.0	0.0	0.0	0.0	0.3	81.9	0.0	0.0	7.0	7.8	3.6

Table 2
Carbon, microcystins, and saxitoxin contents in total seston and in net phytoplankton (20–300 μm) of Funil reservoir related to effects/symptoms observed during the bioassays

Date	Carbon (mg L^{-1})	Microcystins (equivalent MC-LR)		Saxitoxins (equivalent STX)		Effect/symptom
		Seston (μg L^{-1})	Phytopl. (μg g^{-1})	Seston (μg L^{-1})	Phytopl. (μg g^{-1})	
Nov 2002	2.4	1.2	2300	n.a.	b.d.l.	Death; reduced reproduction
Dec 2002	1.4	4.5	1200	n.a.	b.d.l.	Death; reduced reproduction
Jan 2003	0.6	b.d.l.	n.a.	n.a.	n.a.	n.o.t.e.
Feb 2003	2.6	b.d.l.	2500	b.d.l.	82.6	Death; reduced reproduction; paralysis
Mar 2003	0.6	b.d.l.	2500	b.d.l.	6.7	Reduced reproduction; paralysis
Apr 2003	0.6	b.d.l.	1300	n.a.	n.a.	Increased reproduction
May 2003	0.6	b.d.l.	3600	n.a.	27.6	Increased/decreased reproduction
Jun 2003	0.6	b.d.l.	600	n.a.	b.d.l.	n.o.t.e.
Aug 2003	2.2	b.d.l.	3800	b.d.l.	63.5	Death; reduced reproduction; paralysis
Sep 2003	6.5	b.d.l.	3500	b.d.l.	b.d.l.	Increased reproduction
Oct 2003	1.9	b.d.l.	3700	n.a.	b.d.l.	Death; reduced reproduction

n.a. = sample not analyzed; b.d.l. = below detection limit; n.o.t.e. = no observed toxic effect.

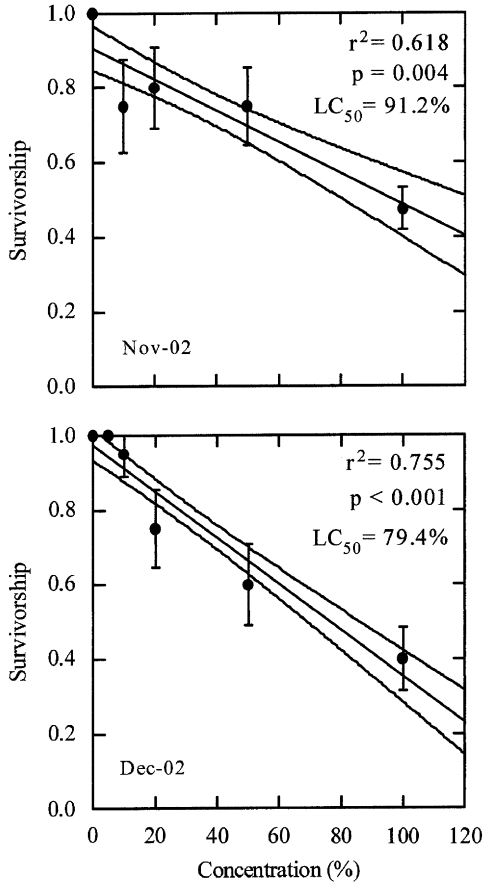

Fig. 3. Acute toxicity bioassays with *Moina micrura* exposed to raw water from Funil reservoir. Curves are regression fits between survivorship and raw water concentration, and ellipses are 95% confidence intervals. Standard error bars are given.

those months because we did not have enough juveniles of this species. Tests with raw water from Lajes reservoir did not reveal any acute toxic effect on cladocerans.

3.3. Chronic bioassays

The chronic bioassays revealed a variety of responses of cladocerans to raw water from Funil reservoir (Figs. 4 and 5; Table 2). Cladocerans showed both decreased and increased reproduction with raw water, paralysis of the swimming movements (in *M. micrura* only), and increased mortality rates. The most deleterious effects were detected during the wet–warm season (November–March). In November the EC_{50} value for *M. micrura* was 21.4% (that is, only 21.4% of raw water was responsible for a 50% reduction in *r* value), and in December this value was only 4.6% (Fig. 4). Mortality rates during those months reached 100% in 100% and 50% of raw water at the end of the experiments, respectively. Experiments with *D. gessneri* started only in January and in this month there was a positive effect of raw water on this cladoceran, with a significant increase in *r* in the two highest concentrations (*t*-test, $p<0.05$) (Fig. 5). In February both cladocerans had a decrease in *r*, with *M. micrura* being more negatively affected ($EC_{50}=36.6\%$) than *D. gessneri* ($EC_{50}=105.2\%$). In March only *M. micrura* was negatively affected in seston concentrations above 20% (*t*-test, $p<0.05$). During the cold–dry season (April–August) there was a decrease in the deleterious effects of raw water on cladocerans, especially on *M. micrura*, which had no effect in April and a significant decrease in *r* only in 100% seston in May (*t*-test, $p=0.041$) (Fig. 4). While *D. gessneri* had a significant decrease in 100% seston in April (*t*-test, $p=0.006$), in May there was a consistent and significant reduction in *r* with increasing raw water concentration for *D. gessneri*, although this effect was small ($EC_{50}=152.1\%$) (Fig. 5). There was a significant (*t*-test, $p<0.05$) decrease in *r* in 100% raw water in June for both cladocerans, although the lower concentrations did not have any effect. In August there was a significant (*t*-test, $p<0.05$) decrease in *r* for both cladocerans, although this effect was small and not proportional to the concentration of raw water in *M. micrura*, while it was for *D. gessneri*. In September (spring), *M. micrura* was positively affected by the raw water, with a significant increase in *r* with increasing raw water concentration. In October both cladocerans were negatively affected by increasing concentration of raw water, with *D. gessneri* being more affected ($EC_{50}=20.3\%$) than *M. micrura* ($EC_{50}=36.3\%$). Mortality rates in October were higher, reaching 100% in raw water concentrations above 25% for *M. micrura*, and in 100% of raw water for *D. gessneri*, at the end of the experiment.

An unexpected effect was observed in February, March, and August, when animals (*M. micrura*) showed paralysis of the swimming movements, staying on the bottom of the test tube. Surprisingly, most of animals survived and even reproduced on the bottom, which was possible because they were able to keep the feeding process through the filtering appendages movement.

Raw water from the Lajes reservoir and filtered water from the Funil reservoir showed no adverse effect on cladocerans reproduction, furnishing indeed high survivorship and fecundity (Fig. 6).

4. Discussion

Ecophysiological effects of MCs in planktonic crustaceans have been reported in several studies, such as reduced filtering rates, growth, and fecundity (Ferrão-Filho and Azevedo, 2003; Lurling, 2003). Biochemical stress in the activity of important enzymes were also reported such as inhibition of phosphatases 1 and 2A (DeMott and Dhawale, 1995; Chen et al., 2005), proteases–trypsin and chemotrypsin (Agrawal et al., 2001, 2005), microsomal glutathione-*S*-transferase (Wiegand et al., 2002), soluble glutathione-*S*-transferase (Beattie et al., 2003), glutathione (Chen et al., 2005), and stimulation of lactate dehydrogenase (Chen et al., 2005). Histological alterations of the gut of *Daphnia* exposed to MCs were also reported (Chen et al., 2005; Rohrlack et al., 2005).

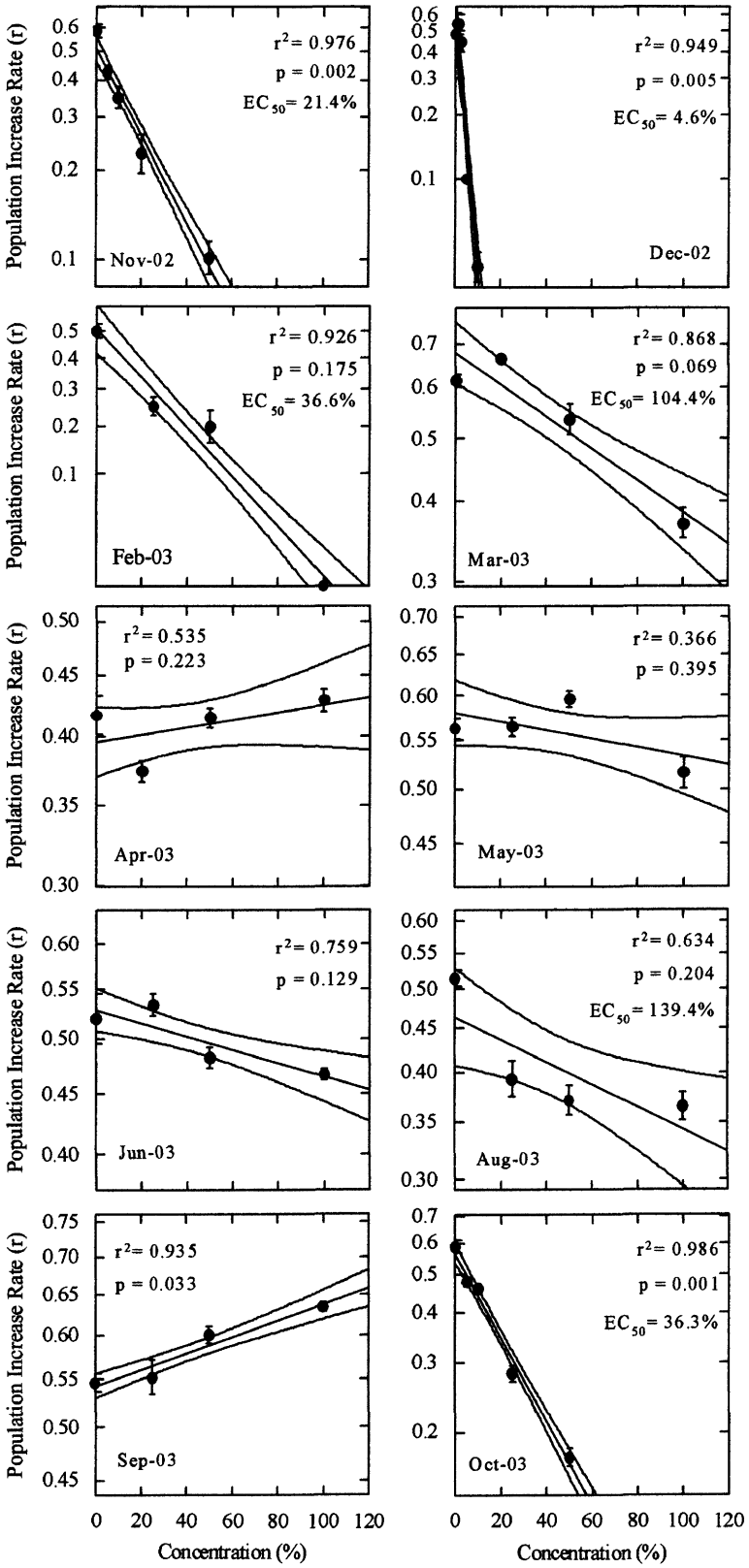

Fig. 4. Chronic toxicity bioassays with *Moina micrura* exposed to raw water from Funil reservoir. Curves are regression fits between the population increase rate (r) and raw water concentration (in %), and ellipses are 95% confidence intervals. Controls are 0 (zero)% raw water (i.e. 100% mineral water). The Y values are given in logarithm scale. Standard error bars are given.

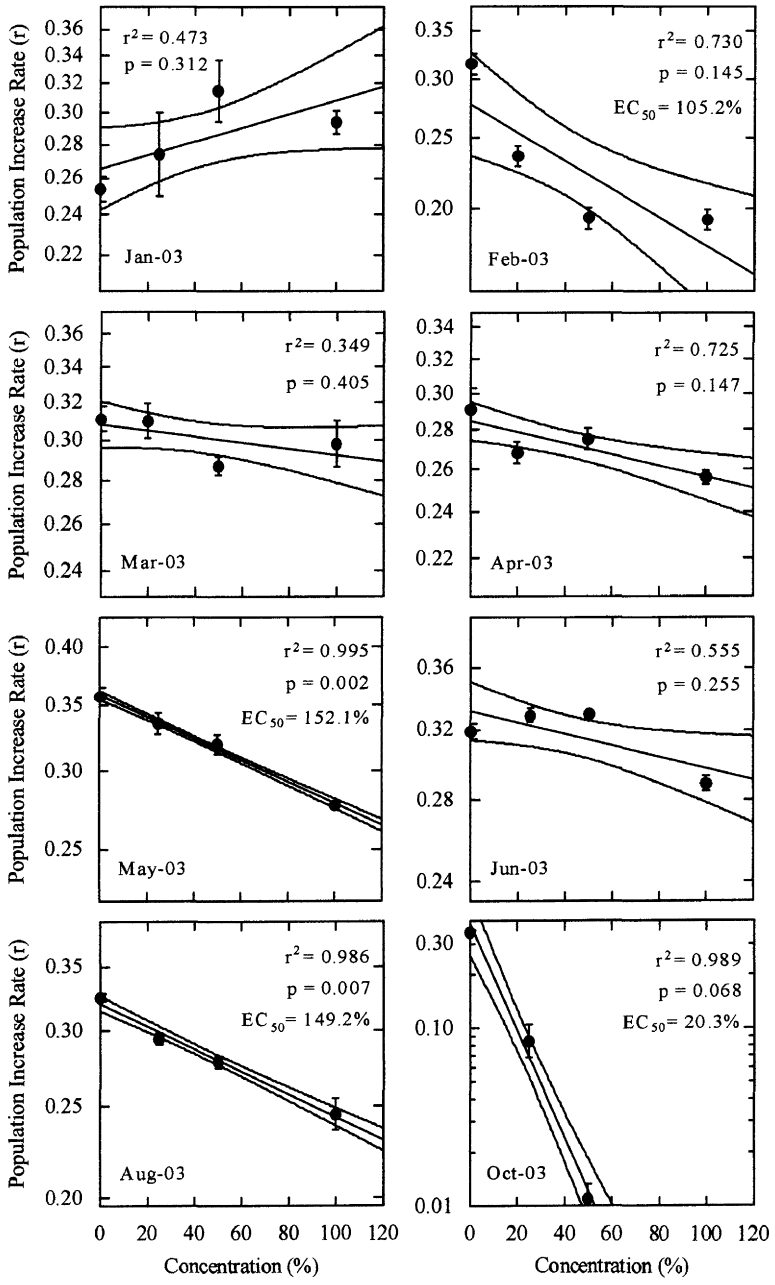

Fig. 5. Chronic toxicity bioassays with *Daphnia gessneri* exposed to raw water from Funil reservoir. Curves are regression fits between the population increase rate (r) and raw water concentration (in %), and ellipses are 95% confidence intervals. Controls are 0 (zero)% raw water (i.e. 100% mineral water). The Y values are given in logarithm scale. Standard error bars are given.

Effects of STXs in zooplankton include a variety of responses including reduced ingestion rates caused by 'physiological incapacitation' (Ives, 1985, 1987), avoidance of toxic cells by chemosensory means in copepods (Huntley et al., 1987; Sykes and Huntley, 1987; Teegarden and Cembella, 1996), reduced somatic growth, size at maturity, and fecundity in copepods (Dutz, 1998; Colin and Dam, 2004). Haney et al. (1995) reported a reduction in the thoracic appendages beating rate and an increased rejection rate of particles by the post-abdomen of *Daphnia carinata* when exposed to a filtrate of *Aphanizomenon flos-aquae* and to purified STX.

Cladoceran toxicity bioassays were shown to be extremely sensitive in detecting toxic effects of cyanobacteria. The responses of cladocerans varied from reduced survivorship and fecundity to paralysis of the swimming movements. Also, responses were more pronounced in months where cyanobacterial biomass and toxin levels were higher. For example, survivorship and reproduction were drastically reduced in the wet–warm season (November–December), when cyanobacteria biomass reached high peaks and MCs were detected in total seston. In October, reproduction was also drastically reduced and there was a high mortality rate in the treatments with

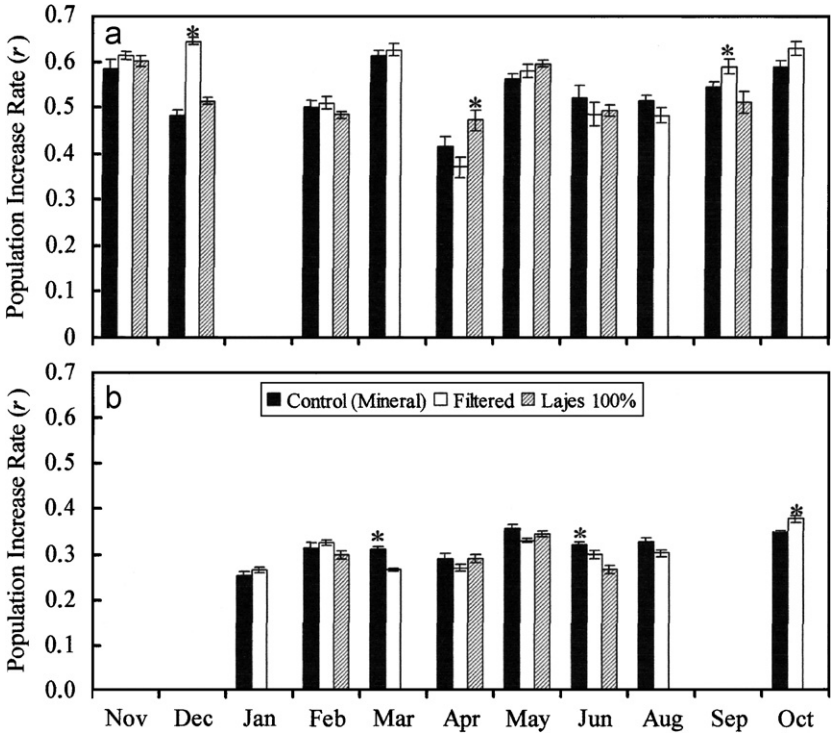

Fig. 6. Population increase rate (r) in controls with mineral water, filtered Funil water, and 100% Lajes raw water. (a) *Moina micrura*; (b) *Daphnia gessneri*. Asterisks indicate significant differences (Student t-test; $p<0.05$). Standard error bars are given.

reservoir water. These months were the periods when *Microcystis* spp. were the dominant cyanobacteria. Some species of this genus are reported to produce the hepatotoxic peptides MCs (Chorus and Bartram, 1999), which are able to reduce survivorship and fecundity of several zooplankton species (DeMott et al., 1991; Ferrão-Filho et al., 2000).

Paralysis of the swimming movements in *M. micrura* was observed in months where *Anabaena* and *Cylindrospermopsis* biomass was high, such as in February, March, and August. Both these cyanobacteria have been reported to produce STXs (Lagos et al., 1999; Landsberg, 2002), which are potent sodium channel blockers, leading to impairing of nerve impulse and muscle paralysis (Adelman et al., 1982; Sasner et al., 1984). Coincidently, STXs were detected in phytoplankton in the same months when paralysis occurred. Similar effects on swimming movements of *D. pulex* and *M. micrura* were reported by Ferrão-Filho et al. (2008) using a STX-producer strain of *C. raciborskii* and raw water from Funil reservoir presenting both potential neurotoxic cyanobacteria. However, those authors attributed the paralysis effect only to *C. raciborskii*, since *Anabaena* spp. was in the form of spiralized trichomes $>100\,\mu m$, which are considered inedible to cladocerans (Arnold, 1971; Porter and Orcutt, 1980; de Bernardi and Giussani, 1990). Although *C. raciborskii* was always present as straight filaments of 100–300 μm in length, the filaments are very friable and animals must be able to ingest their fragments.

Filtered water from Funil reservoir did not cause any toxic effect on cladocerans and, when supplemented with good food (i.e., green algae), furnished high population growth rates, indicating no presence of toxic chemicals in solution and suggesting that cyanotoxins in the particulate fraction may be responsible for the toxic effect observed with the raw water sample. Therefore, cyanobacteria in seston were probably responsible for the toxic effects observed in cladocerans.

STXs in the few seston samples analyzed were below the detection limit, while net concentrated phytoplankton presented high levels of MCs in all months and low levels of STXs in some samples. STXs, however, could be detected only in phytoplankton samples concentrated by net hauls, in months when *C. raciborskii* biomass was high. Nevertheless, even when MC and STX concentrations are low, they may accumulate in zooplankton (Thostrup and Christoffersen, 1999; Ferrão-Filho et al., 2002; Chen et al., 2005; Teegarden and Cembella, 1996; Hamazaki et al., 2003; Nogueira et al., 2004) and may exceed threshold concentrations and become harmful to these organisms (Chen et al., 2005).

Cyanotoxins detected in seston were likely to have caused the reductions in survivorship and fecundity of cladocerans. These reductions may be the result of a synergistic effect of the toxins in combination or may be a result of one toxin acting predominantly over the other, since some months presented higher levels of one toxin relative to the other. Also, the mechanisms of action of

these toxins are different. While MCs act as a phosphatase 1 and 2A inhibitor, STXs act by blocking sodium channels, leading to disruption of normal nerve impulse (Carmichael, 1992). In months in which potential MC-producer cyanobacteria were dominant in phytoplankton, animals showed high mortality rates, besides decreased reproduction. In months when potential STX-producer cyanobacteria species were dominant, animals showed almost immediate paralysis (noted after 24 h) in chronic tests, presenting, however, relatively good survivorship and reproduction. An exception was February, when reduced survivorship and fecundity were observed at the same time as the paralysis of animals, which was probably related to high levels of STXs in phytoplankton.

Another unexpected exception was observed in September, when *M. aeruginosa* dominated and animals showed increased reproduction with increasing concentration of raw water. This may be explained by the fact that *Microcystis* was present mostly as colonies of big size ($>100\,\mu m$) in this month, being thus likely inedible to cladocerans, despite the high biomass of cyanobacteria and high concentration of MCs. Another explanation is that the increasing amounts of reservoir water likely improved the water quality, increasing dissolved salts and organic compounds (e.g. vitamins), while other factors (e.g. food, as green algae) remained constant, and toxicity was negligible because of the inedible size of *Microcystis* colonies. Further, bacteria associated with high cyanobacterial biomass may be a good food source for cladocerans (Hanazato, 1991). The lack of effect in April and May, when other phytoplankton groups dominated over cyanobacteria, corroborates the fact that cyanobacteria can have decreased toxicity when mixed with a sufficient amount of good food (Ferrão-Filho et al., 2000).

The possibility that other unidentified toxins in seston from Funil reservoir have caused the observed effects in cladocerans cannot be disregarded. Ferrão-Filho et al. (2008) have found some inhibition of acetylcholinesterase (AChE) activity in seston from Funil reservoir in August 2003, when *Anabaena* comprised a great part of phytoplankton biomass. Anatoxin-*a*(s), produced by some *Anabaena* spp., is known to inhibit AChE irreversibly (Carmichael, 1992). However, since the inhibition effect was small (28%) and animals showed almost complete recovery of mobility after 48 h when removed from raw water, the paralysis effect was likely caused by STXs.

Sensitivity to toxic cyanobacteria may vary among zooplankton species and is a function of the ingestion and digestion capacity of single cells, colonies, or filaments (Ferrão-Filho and Azevedo, 2003), of the susceptibility to toxins as dissolved or cell-bound toxins (DeMott et al., 1991), and of the resistance of the zooplankton species to toxins (Colin and Dam, 2004). It has been hypothesized that tropical zooplankton species would be more resistant to cyanotoxins since these species live in more eutrophicated environments and have a longer history of coexistence with cyanobacteria than temperate species (Nandini and Rao, 1998; Ferrão-Filho et al., 2000). Our results contradicted this hypothesis, since both species exhibited sensitivity to cyanobacteria present in Funil reservoir water. However, *M. micrura* seems to be more sensitive than *D. gessneri* since it was more affected by Funil raw water. Also, while the former species showed paralysis in some months, the latter never showed this symptom. In the study of Ferrão-Filho et al. (2008) *M. micrura* was shown to be extremely sensitive to STXs, being immobilized at concentrations as low as $0.94\,ng\,eq\,STX\,L^{-1}$, while *D. gessneri* was not affected even in the highest toxin concentration ($9.37\,ng\,eq\,STX\,L^{-1}$). In this study, similar effects were observed in *M. micrura* (but not in *D. gessneri*), mainly when potentially STX-producer cyanobacteria and higher amounts of STXs occurred. Thus, these results suggest that *D. gessneri* is highly resistant to STXs, and that this difference on toxin resistance can result in an important change on zooplanktonic community structure in eutrophic reservoirs.

5. Conclusions

In conclusion, our results are consistent with toxic effects of cyanobacteria on zooplankton species also found in other studies, such as high mortality rates, when MCs are present in an edible size fraction of phytoplankton, and paralysis of swimming movements, when STXs are present. These results also suggest the potential use of native cladoceran species as sensitive model organisms in toxicity bioassays and in biomonitoring of cyanotoxins in highly eutrophicated reservoirs dominated by cyanobacteria.

Acknowledgments

We thank Jobson M. Barbosa and Ricardo F.G. de Souza for the field sampling and microcystins analysis, and Andrea A. Gomes for saxitoxin analysis, from the Laboratory of Ecophysiology and Toxicology of Cyanobacteria, at Federal University of Rio de Janeiro, and Ricardo Mugnai from the Laboratory of Evaluation and Promotion of Environmental Health, at Instituto Oswaldo Cruz, FIOCRUZ, for the help with graphics. We also thank Dr. Alan J. Tessier from the National Science Foundation for providing the strain of the green algae *A. falcatus*.

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